

REPORT DOCUMENTATION PAGE		Form Approved OMB NO. 0704-0188	
Public Reporting Burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comment regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington DC 20503			
1. AGENCY USE ONLY (Leave Blank)		2. REPORT DATE:	
		3. REPORT TYPE AND DATES COVERED Final Report 1-Jun-2005 - 31-May-2005	
4. TITLE AND SUBTITLE Biological Sensing with Terahertz Circular Dichroism Spectroscopy		5. FUNDING NUMBERS DAAD190210080	
6. AUTHORS Kevin W. Plaxco, S. James Allen		8. PERFORMING ORGANIZATION REPORT NUMBER	
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES University of California - Santa Barbara Office of Research 3227 Cheadle Hall Santa Barbara, CA 93106 -2050			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211		10. SPONSORING / MONITORING AGENCY REPORT NUMBER 42628-EL.2.00	
11. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.			
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) The abstract is below since many authors do not follow the 200 word limit			
14. SUBJECT TERMS Terahertz, sub-millimeter, circular dichroism, bw detection		15. NUMBER OF PAGES Unknown due to possible attachments	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION ON THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL

ABSTRACT

We have developed a circular dichroism spectrometer working in the terahertz (THz) frequency regime. As THz spectroscopy is specific to collective vibrational modes in macromolecules (and collective modes in condensed, polar media such as water), the spectrometer should provide spectral fingerprints of biological materials. Moreover, by focusing on circular dichroism the spectrometer should provide fingerprints of these materials uncontaminated by the strong background absorbance of water and other polar materials. We have built this spectrometer and have pushed its signal to noise down to 1 part in 10^4 . While this is more than sufficient to detect the strong circular dichroism of macroscopic, chiral objects, it is apparently insufficient to observe circular dichroism arising from common biological materials. Current efforts are aimed at 1-2 orders of magnitude improvements on this detection limit.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Xu, J., Ramian, G.J., Galan, J.F., Savvidis, P.G., Scopatz, A.M., Birge, R.R., Allen, S.J. and Plaxco, K.W. (2003) "Terahertz circular dichroism spectroscopy: a potential approach to unbiased, in situ life detection." *Astrobiology*, 3, 489-504

Number of Papers published in peer-reviewed journals: 1.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Xu, J., Ramian, G.J., Galan, J.F., Savvidis, P.G., Scopatz, A.M., Birge, R.R., Allen, S.J. and Plaxco, K.W. (2004) "Terahertz circular dichroism spectroscopy of biomolecules." In: *Chemical and Biological Standoff Detection*. J.O. Jensen and J-M. Theriault, Eds. Proceedings of the SPIE, Vol. 5268, pp 19-26

Number of Papers published in non peer-reviewed journals: 1.00

(c) Papers presented at meetings, but not published in conference proceedings (N/A for none)

Plaxco, K.W. "Terahertz circular dichroism as an unbiased, in situ life detection technology." American Geophysical Society Fall Meeting, San Francisco, CA, 12/07/2

Xu, J. "Terahertz circular dichroism of macroscopic, chiral objects and biopolymers." Am. Phys. Soc, Montreal, Canada, 3/04

Number of Papers not Published: 2.00

(d) Manuscripts

Xu, J., Plaxco, K.W. and Allen, S.J. (2005) "Absorption spectra of liquid water and aqueous buffers between 0.3 - 3.72 terahertz." *J. Chem. Phys.* Submitted.

Number of Manuscripts: 1.00

Number of Inventions:

Graduate Students

Jing Xu. 49%

Number of Graduate Students supported: 1.00

Total number of FTE graduate students: 0.00

Names of Post Doctorates

Savvidis, P.G 25%

Number of Post Docs supported: 1.00
Total number of FTE Post Doctorates: 0.00

List of faculty supported by the grant that are National Academy Members

Names of Faculty Supported

Kevin W. Plaxco
S. James Allen
Number of Faculty: 2.00

Names of Under Graduate students supported

Scopatz, A.
Number of under graduate students: 1.00

Names of Personnel receiving masters degrees

Number of Masters Awarded: 0.00

Names of personnel receiving PHDs

Number of PHDs awarded: 0.00

Names of other research staff

Jerry Ramian

Sub Contractors (DD882)

1 a. 1 b.

Sub Contractor Numbers (c):
Patent Clause Number (d-1):
Patent Date (d-2):
Work Description (e):
Sub Contract Award Date (f-1):
Sub Contract Est Completion Date(f-2):

Inventions (DD882)

5

Patent Filed in US? (5d-1)

Patent Filed in Foreign Countries? (5d-2)

Was the assignment forwarded to the contracting officer? (5e)

Foreign Countries of application (5g-2):

5a:

5f-1a:

5f-c:

Closing Technical Report for “Submillimeter-wave Circular Dichroism Spectroscopy of Biomaterials in Water”

PIs S. James Allen, Kevin W. Plaxco; University of California, Santa Barbara

List of Appendixes Illustrations and Tables: .

Xu *et al.*, 2005 is attached as an appendix

Statement of the problem studied We have characterized both the absorbance and circular dichroism spectroscopy of several characteristic biopolymers under realistic (aqueous) conditions. All previous studies of the THz absorbances of these materials were conducted on dry samples so as to avoid the strong absorbance of water, but these samples are poor proxies for the solution-phase biomolecules most likely to be encountered in BW detection scenarios.

Summary of the most important results In order to overcome the strong THz absorbance produced by liquid water we have employed two distinct and yet complimentary approaches. The first was to conduct direct absorbance measurements using the kW power of the UCSB free electron laser (FEL) coupled with highly sensitive, cryogenic detectors. This combination allows us to probe even extremely thick, optically dense samples. We also designed and optimized several variable length sample cells that allow us to extract the absorbance of solution-phase biomolecules against a background of much more strongly absorbing material. Key elements of this approach involve the automated collection of data over a range of pathlengths. This allows us to determine extinction coefficients from knowledge of how absorbance changes with pathlength, obviating the need to know either absolute absorbance or absolute pathlength and greatly simplifying the requisite measurements (for example, the inevitable absorbance and reflectance of the sample cell do not contribute to the difference signal and are thus quite effectively controlled for). Using this approach we have measured the absorbance spectra of bovine serum albumin and lysozyme in water (manuscripts in preparation). We have also conducted the most precise and exhaustive survey of the absorbance

spectrum of water (and several aqueous buffers) over the range 0.3 to 3.4 THz (Xu *et al.*, 2005).

Our second approach for overcoming the strong THz absorbance of water was to perform circular dichroism spectroscopy. Simulations and analytical calculations suggest that the CD spectrum of a protein/DNA in the THz regime will be ~ 3 to 4 orders of magnitude reduced from the absorbance spectrum (theory developed in Xu *et al.*, 2003; 2004). Water, however, is achiral and thus does not exhibit CD features. Thus, under the auspices of this grant we built and characterized several circular dichroism spectrometer architectures. In particular we discovered that our initial architecture, which generated circular dichroism using quartz crystal 1/4 wave plates, suffered from severe etalon effects producing enormous, artifactual circular dichroism. Realizing this we set out to build, debug and characterize an interferometric-based CD spectrometer. Through careful adjustment of the length of the interferometer arms we are able to accurately null the spectrometer to produce background CD of less than 1 part in 6000. Unfortunately no CD was apparent in test biological samples at that level of signal to noise. We have since improved the s/n and are currently measuring the TCD spectra of several representative biomolecules.

Bibliography

Xu, J., Ramian, G.J., Galan, J.F., Savvidis, P.G., Scopatz, A.M., Birge, R.R. Allen, S.J. and Plaxco, K.W. (2003) "Terahertz circular dichroism spectroscopy: a potential approach to unbiased, *in situ* life detection." *Astrobiology*, **3**, 489-504

Xu, J., Ramian, G.J., Galan, J.F., Savvidis, P.G., Scopatz, A.M., Birge, R.R., Allen, S.J. and Plaxco, K.W. (2004) "Terahertz circular dichroism spectroscopy of biomolecules." In: Chemical and Biological Standoff Detection. J.O. Jensen and J-M. Theriault, Eds. Proceedings of the SPIE, Vol. **5268**, pp 19-26

Xu, J., Plaxco, K.W. and Allen, S.J. (2005) "Absorption spectra of liquid water and aqueous buffers between 0.3 - 3.72 terahertz." *J. Chem. Phys.* Submitted.

Appendix I: Xu et al., 2005

Absorption spectra of liquid water and aqueous buffers between 0.3 - 3.72 terahertz

Jing Xu,^{a)} Kevin W. Plaxco,^{b)} and S. James Allen^{a)}

^{a)} Department of Physics, University of California, Santa Barbara, CA 93106

^{b)} Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106

(Received

Abstract: We have developed a terahertz absorption spectrometer suitable for strongly absorbing liquids such as water, and have precisely measured the absorption spectrum of water between 0.3 – 3.72 THz ($10 - 124 \text{ cm}^{-1}$). We have also examined the absorption spectra of aqueous potassium phosphate buffers at pH 3 and 8, and find that they do not differ significantly from pure distilled deionized water.

Keywords: far infrared, free electron laser, sub-millimeter wave, terahertz, water.

PACS number: 33.20.-t, 33.20.Ea, 61.25.-f, 61.25.Em, 82

I. INTRODUCTION

Water exhibits strong frequency dependent terahertz absorption with attenuation lengths on the order of tens of micrometers. To access this spectral information, previous experimental investigations have employed femtosecond time domain transmission¹ and reflection² spectroscopy, reflection dispersive Fourier transform spectroscopy^{3,4}, optically pumped far infrared (FIR) laser transmission spectroscopy⁵⁻⁷, and FIR grating transmission measurements⁸. Below ~ 1.5 THz, they provide a well documented absorption spectrum for liquid water, at approximately room temperature. At higher frequencies the published data⁴⁻⁸ is comparatively sparse, and the agreement is less satisfying. In particular there are wide discrepancies among the transmission measurements with optically pumped FIR lasers⁵⁻⁷. Analysis by Vij et al.⁵ suggests that multiple reflections between the sample cell windows (standing wave or etalon effect⁹) can contribute significantly to uncertainties in measured terahertz absorption coefficients.

Here we have eliminated the standing wave artifact and measured the terahertz absorption of distilled deionized water over the frequency range of 0.3 – 3.72 THz ($10 - 124 \text{ cm}^{-1}$) with improved accuracy. We have also measured the terahertz absorption spectra of aqueous potassium phosphate buffers at pH 3 and 8.

II. METHODS AND MATERIALS

A. Methods

We have developed a terahertz absorption spectrometer suitable for studying strongly absorbing liquids such as water. Our spectrometer combines kilowatt-power terahertz radiation from the UCSB free-electron lasers with an ultra-sensitive cryogenic detector, and is able to withstand attenuation of more than five orders of magnitude. A

reference pyroelectric detector is employed to monitor the fluctuations in incident laser power. A precision variable path length cell^{1,10}, accurate to $\sim 0.5 \mu\text{m}$, allows for reproducible control of the sample thickness. The cell windows are made of high density polyethylene, which is highly transparent and has an index of refraction that closely matches that of liquid water over the frequency range of interest¹¹.

We precisely determined the absorption coefficients of water using linear fits of the change in absorbance, ΔA , with changing path length, Δl ,

$$\Delta A = \Delta \left(-\ln \left[\frac{I_t}{I_{reference}} \right] \right) = \alpha \cdot \Delta l ,$$

where I_t is the transmitted intensity, and $I_{reference}$ removes fluctuations in the incident radiation. We carefully choose the smallest path length at each frequency to ensure at least three orders of magnitude of attenuation. This, together with the closely matched indexes of refraction of cell window and liquid water, eliminate any standing wave artifact that has apparently contributed to uncertainties in some of the previous measurements⁵⁻⁷. This approach also obviates the need to precisely characterize the optical properties of the cell window, and the need for precise determination of the absolute path lengths.

B. Materials

Water samples were prepared at 22°C using distilled deionized water, and distilled deionized water buffered at pH 3 and 8 with 50 mM potassium phosphate.

III. RESULTS AND DISCUSSION

We measured the change in the terahertz absorbance of water with changing path length (Fig. 1). At each wavelength, we examined nine distinct path lengths, with

increments ranging between 2 - 20 μm depending on the attenuation strength of the sample, and repeated the absorbance measurements approximately eight times to estimate confidence limits.

The approach we have adopted here does not require the precise knowledge of either the absolute path length or the optical properties of the sample cell, both of which are difficult to access and can contribute to error in final absorption measurements. Instead we employ only two variables, ΔA and Δl , which are readily obtained with high precision with the detectors and translation stage we have chosen. Standing waves artifacts that may have contributed to uncertainties in previous investigations⁵⁻⁷ are not observed in our measurements, allowing us to precisely determine the absorption coefficients from least squares fits of the change in absorbance observed with changing in path length (Fig. 1).

We have precisely measured the absorption spectra of distilled deionized water, as well as aqueous phosphate buffers at pH 3 and 8, over the frequency range of 0.3 - 3.72 THz. We do not observe any statistically significant differences in the absorption of deionized water and the two aqueous buffers (Fig. 2). This is, for us, somewhat surprising as we have expected the terahertz absorption of water to depend sensitively on its ionic content. Nevertheless, our finding may prove to be a useful reference for solvent subtraction in future absorption study of buffered biomolecules in aqueous solutions.

Below ~ 1.5 THz our absorption data is in excellent agreement with the published data¹⁻⁸. Above this frequency, however, we observe statistically significant discrepancies between our observations and those previously reported using optically pumped FIR laser transmission spectroscopy⁵⁻⁷, and FIR grating measurements⁸ (Fig. 3). The discrepancies

among the previous transmission measurements have been suggested to arise from the formation of standing waves between the sample cell windows⁵. We have eliminated this potentially important artifact by maintaining a minimum path length that strongly attenuates the incident terahertz radiation (> 1000 times), and by closely matching the index of refraction of our cell windows with that of water. Thus, in addition to including several previously unexplored frequencies above 1.5 THz, our observations provide continuing and perhaps more accurate documentation for water absorption at higher terahertz frequencies up to 3.72 THz (Table I).

ACKNOWLEDGEMENTS

We acknowledge the support from Army Research Office through grants DAAD19-02-01-0080 and W911NF0510034, NASA through grant NAG5-12150.

REFERENCES

- ¹J. T. Kindt, and C. A. Schmittenmaer, J. Phys. Chem. **100**, 10373 (1996).
- ²L. Thrane, R. H. Jacobsen, P. Uhd Jepsen, and S. R. Keiding, Chem. Phys. Lett. **240**, 330 (1995).
- ³J. B. Hasted, S. K. Husain, F. A. M. Frescura, and J. R. Birch, Chem. Phys. Lett. **118**, 622 (1985).
- ⁴M. N. Afsar, and J. B. Hasted, J. Opt. Soc. Am. **67**, 902 (1977).
- ⁵J. K. Vij, Int. J. Infrared Milim. Waves **10**, 847 (1989).
- ⁶G. J. Evans, M. W. Evans, P. Minguzzi, G. Salvetti, C. J. Reid, and J. K. Vij, J. Mol. Liq. **34**, 285, (1987).
- ⁷O. A. Simpson, B. L. Bean, and S. Perkowitz, J. Opt. Soc. Am. **69**, 1723, (1979).
- ⁸C. W. Robertson, B. Curnutte, and D. Williams, Mol. Phys. **26**, 183 (1973).
- ⁹G. R. Fowles, Introduction to Modern Optics, 2nd ed. (Dover, New York 1975), p. 86.
- ¹⁰A. J. Fitzgerald, E. Berry, N. N. Zinov'ev, S. Homer-Vanniasinkam, R. E. Miles, J. M. Chamberlain, and M. A. Smith, J. Biol. Phys. **29**, 123 (2003).
- ¹¹D. R. Smith, and E. V. Loewenstein, Appl. Opt. **14**, 1335 (1975).

TABLE I. The measured absorption coefficients of liquid water over 0.3 - 3.72

THz, errors are enclosed in parenthesis.

Frequency	Absorption Coefficients	Frequency	Absorption Coefficients
(THz)	(cm ⁻¹)	(THz)	(cm ⁻¹)
0.30	123 (2)	1.40	275 (2)
0.45	148 (4)	1.53	294 (2)
0.48	156 (1)	1.56	295 (1)
0.60	167 (1)	1.83	326 (6)
0.66	180 (1)	1.98	347 (7)
0.90	210 (1)	2.13	367 (5)
0.99	220 (3)	2.52	433 (7)
1.05	237 (1)	2.84	502 (13)
1.26	256 (2)	3.42	622 (6)
1.29	269 (1)	3.72	739 (21)
1.31	271 (1)		

FIG. 1. The absorption coefficients of water are precisely determined from linear fits of the change in absorbance with changing path length. We repeat the absorbance measurements approximately eight times to estimate our confidence level. We need not consider the absolute path length, or the intrinsic optical properties of our cell window. Standing waves artifact is not present in our measurements, allowing for accurate determination of absorption coefficients.

FIG. 2. (Color in Printed Journal) The terahertz absorption of distilled deionized water does not differ significantly from that of aqueous phosphate buffers (50 mM) at pH 8 and pH 3.

FIG. 3. (Color in Printed Journal) Previously published water absorption spectra include measurements using femtosecond time domain transmission¹ (∇) and reflection² (\otimes) spectroscopy, reflection dispersive Fourier transform spectroscopy^{3,4} (O, and \oplus respectively), optically pumped FIR laser transmission spectroscopy⁵⁻⁷ (Δ , \diamond , Θ), and FIR grating spectroscopy⁸ (\square). Our data is in excellent agreement with the published data below ~ 1.5 THz. Above this frequency, our measurements provide continuing and perhaps more accurate documentation for water absorption up to 3.72 THz.

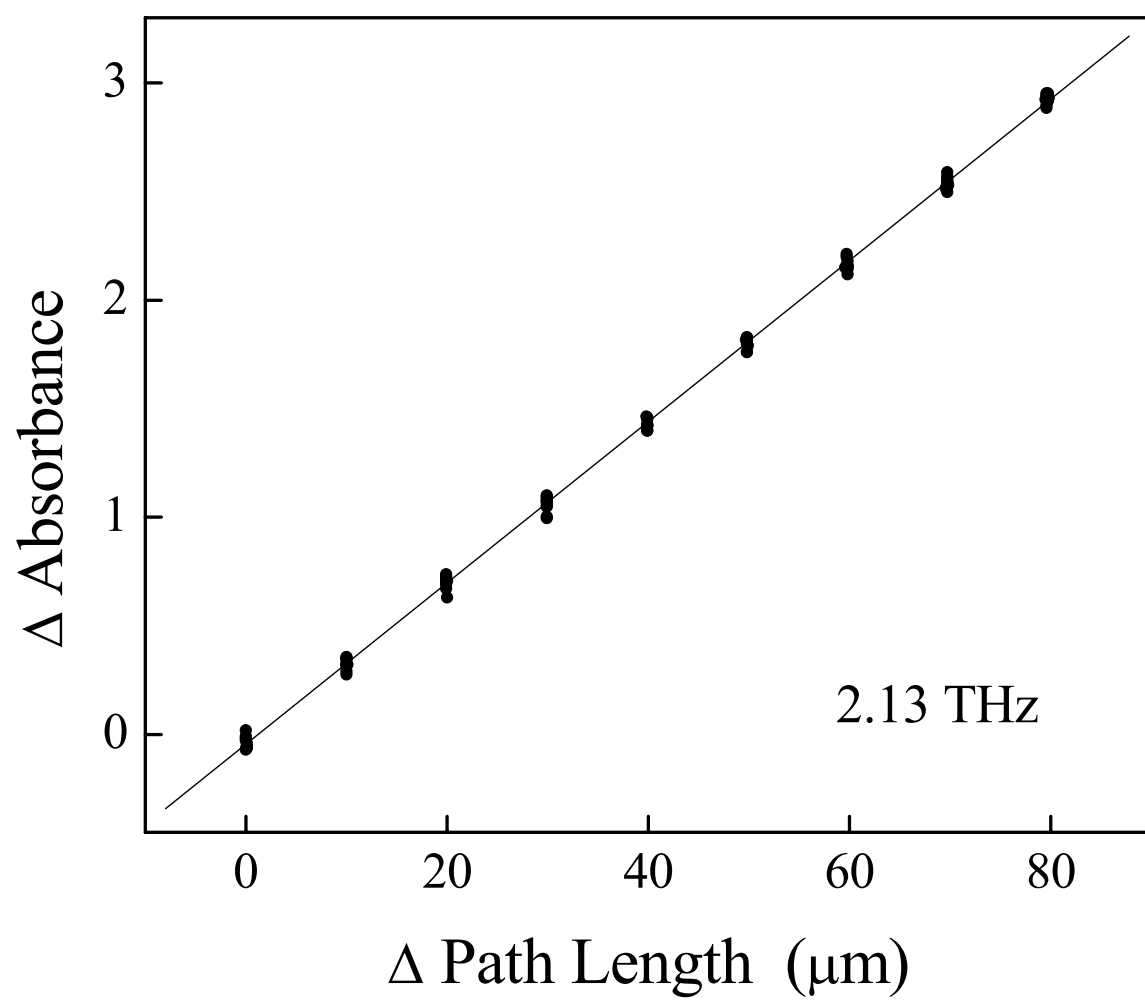


FIG. 1. Xu et. al.

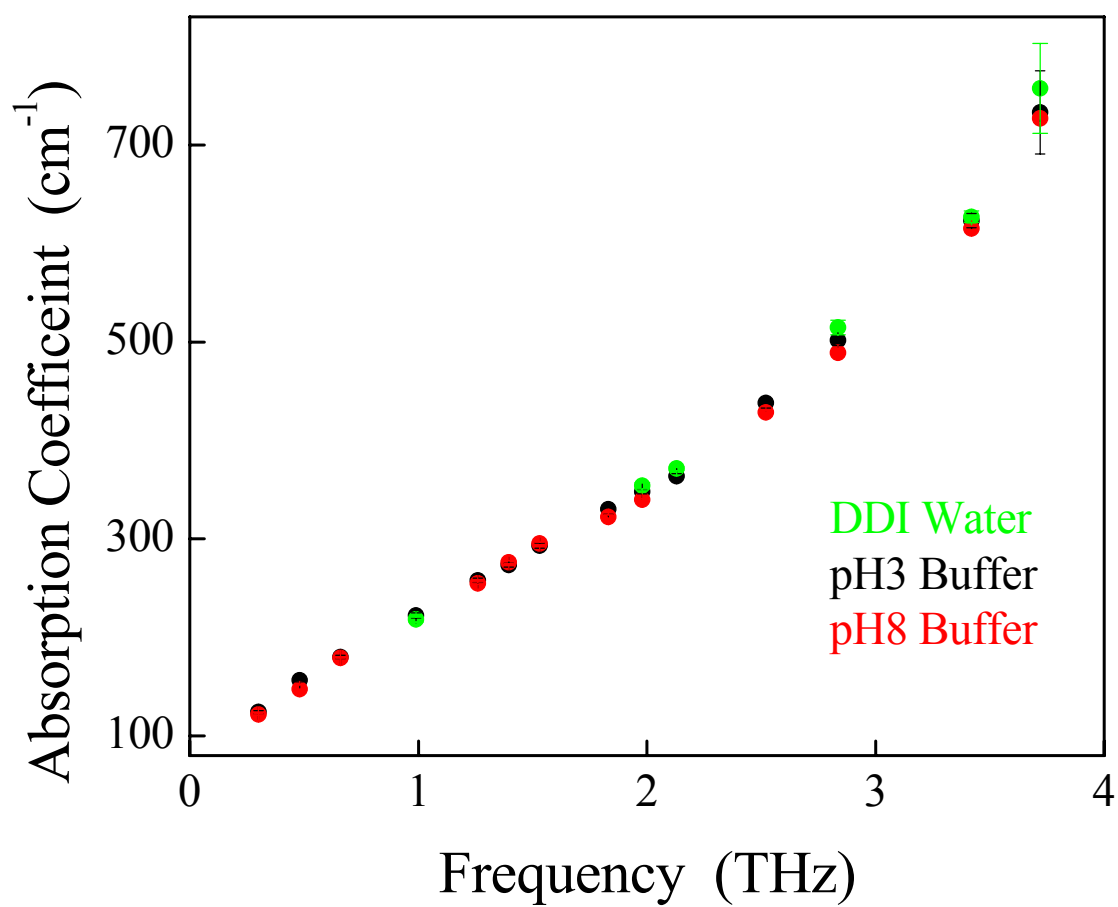


FIG. 2. Xu et. al.

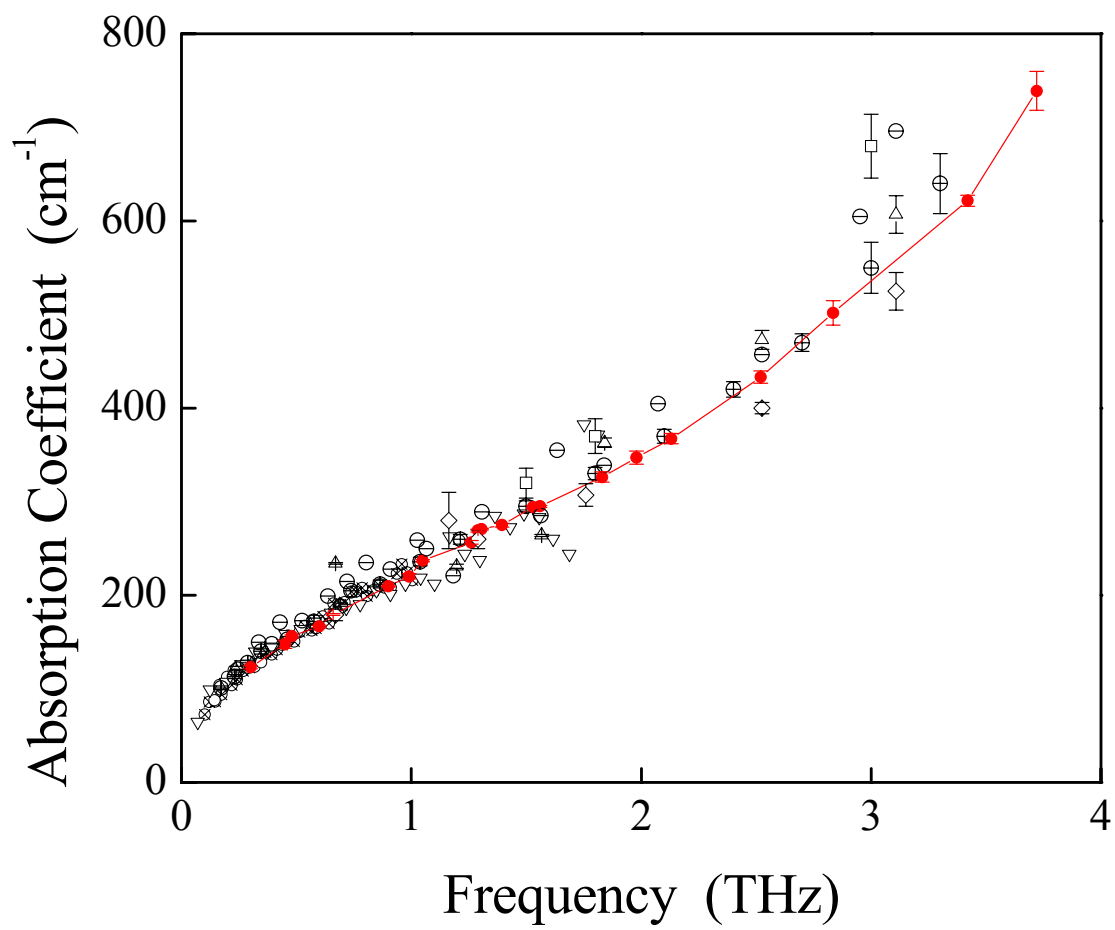


FIG. 3. Xu et al.